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(54) Title: METHOD AND APPARATUS FOR FABRICATING MICROARRAYS OF BIOLOGICAL SAMPLES

(57) Abstract

A method and apparatus for forming microarrays of biological samples on a support are disclosed. The method involves dispensing a known volume of a reagent at each of a selected array position, by tapping a capillary dispenser on the support under conditions effective to draw a defined volume of liquid onto the support. The apparatus is designed to produce a microarray of such regions in an automated fashion.

METHOD AND APPARATUS FOR FABRICATING MICROARRAYS OF BIOLOGICAL SAMPLES

Field of the Invention

This invention relates to a method and apparatus for fabricating microarrays of biological samples for large scale screening assays, such as arrays of DNA samples to be used in DNA hybridization assays for genetic research and diagnostic applications.

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In addition, the number of arrays that can be made with each dipping is usually quite small.

An alternate method of creating ordered arrays of nucleic acid sequences is described by Pirrung, *et al.* (1992), and also by Fodor, *et al.* (1991). The method involves synthesizing different nucleic acid sequences at different discrete regions of a support. This method employs elaborate synthetic schemes, and is generally limited to relatively short nucleic acid sample, *e.g.*, less than 20 bases. A related method has been described by Southern, *et al.* (1992).

Khrapko, *et al.* (1991) describes a method of making an oligonucleotide matrix by spotting DNA onto a thin layer of polyacrylamide. The spotting is done manually with a micropipette.

None of the methods or devices described in the prior art are designed for mass fabrication of microarrays characterized by (i) a large number of micro-sized assay regions separated by a distance of 50-200 microns or less, and (ii) a well-defined amount, typically in the picomole range, of analyte associated with each region of the array.

Furthermore, current technology is directed at performing such assays one at a time to a single array of DNA molecules. For example, the most common method for performing DNA hybridizations to arrays spotted onto porous membrane involves sealing the membrane in a plastic bag (Maniatas, *et al.*, 1989) or a rotating glass cylinder (Robbins Scientific) with the labeled hybridization probe inside the sealed chamber. For arrays made on non-porous surfaces, such as a microscope slide, each array is incubated with the labeled hybridization probe sealed under a coverslip. These techniques require a separate sealed chamber for

reagents in each well without cross-contamination. How ver, each well is intended to hold only one target element whereas the invention described here makes a microarray of many biomolecules in each subdivided region of the solid support. Furthermore, the 96 well plates are at least 1 cm thick and prevent the use of the device for many colorimetric, fluorescent and radioactive detection formats which require that the membrane lie flat against the detection surface. The invention described here requires no further processing after the assay step since the barriers elements are shallow and do not interfere with the detection step thereby greatly increasing convenience.

Hyseq Corporation has described a method of making an "array of arrays" on a non-porous solid support for use with their sequencing by hybridization technique. The method described by Hyseq involves modifying the chemistry of the solid support material to form a hydrophobic grid pattern where each subdivided region contains a microarray of biomolecules. Hyseq's flat hydrophobic pattern does not make use of physical blocking as an additional means of preventing cross contamination.

Summary of the Invention

The invention includes, in one aspect, a method of forming a microarray of analyte-assay regions on a solid support, where each region in the array has a known amount of a selected, analyte-specific reagent. The method involves first loading a solution of a selected analyte-specific reagent in a reagent-dispensing device having an elongate capillary channel (i) formed by spaced-apart, coextensive elongate members, (ii) adapted to hold a quantity of the reagent solution and (iii) having a tip region at which aqueous

s lected volume on the support, e.g., a selected volume in the volume range 0.01 to 100 nl.

The positioning and dispensing structures are controlled by a control unit in the apparatus. The unit operates to (i) place the dispensing device at a loading station, (ii) move the capillary channel in the device into a selected reagent at the loading station, to load the dispensing device with the reagent, and (iii) dispense the reagent at a defined array position on each of the supports on said holder. The unit may further operate, at the end of a dispensing cycle, to wash the dispensing device by (i) placing the dispensing device at a washing station, (ii) moving the capillary channel in the device into a wash fluid, to load the dispensing device with the fluid, and (iii) remove the wash fluid prior to loading the dispensing device with a fresh selected reagent.

The dispensing device in the apparatus may be one of a plurality of such devices which are carried on the arm for dispensing different analyte assay reagents at selected spaced array positions.

In another aspect, the invention includes a substrate with a surface having a microarray of at least 10^3 distinct polynucleotide or polypeptide biopolymers in a surface area of less than about 1 cm^2. Each distinct biopolymer (i) is disposed at a separate, defined position in said array, (ii) has a length of at least 50 subunits, and (iii) is present in a defined amount between about 0.1 femtomoles and 100 nanomoles.

In one embodiment, the surface is glass slide surface coated with a polycationic polymer, such as polylysine, and the biopolymers are polynucleotides. In another embodiment, the substrate has a water-impermeable backing, a water-permeable film formed on

representing a plurality of known genes derived from the two cell types, under conditions that result in hybridization of the cDNA's to complementary-sequence polynucleotides in the array. The array is then examined by fluorescence under fluorescence excitation conditions in which (i) polynucleotides in the array that are hybridized predominantly to cDNA's derived from one of the first and second cell types give a distinct first or second fluorescence emission color, respectively, and (ii) polynucleotides in the array that are hybridized to substantially equal numbers of cDNA's derived from the first and second cell types give a distinct combined fluorescence emission color, respectively. The relative expression of known genes in the two cell types can then be determined by the observed fluorescence emission color of each spot.

These and other objects and features of the invention will become more fully apparent when the following detailed description of the invention is read in conjunction with the accompanying figures.

Brief Description of the Drawings

Fig. 1 is a side view of a reagent-dispensing device having a open-capillary dispensing head constructed for use in one embodiment of the invention;

Figs. 2A-2C illustrate steps in the delivery of a fixed-volume bead on a hydrophobic surface employing the dispensing head from Fig. 1, in accordance with one embodiment of the method of the invention;

Fig. 3 shows a portion of a two-dimensional array of analyte-assay regions constructed according to the method of the invention;

Fig. 4 is a planar view showing components of an aut mated apparatus for forming arrays in accordance with the inventi n.

arrays of M13 clones in each of four quadrants, where each quadrant was hybridized simultaneously to a different oligonucleotide using an open face hybridization method.

Detailed Description of the Invention

I. Definitions

Unless indicated otherwise, the terms defined below have the following meanings:

"Ligand" refers to one member of a ligand/anti-ligand binding pair. The ligand may be, for example, one of the nucleic acid strands in a complementary, hybridized nucleic acid duplex binding pair; an effector molecule in an effector/receptor binding pair; or an antigen in an antigen/antibody or antigen/antibody fragment binding pair.

"Antiligand" refers to the opposite member of a ligand/anti-ligand binding pair. The antiligand may be the other of the nucleic acid strands in a complementary, hybridized nucleic acid duplex binding pair; the receptor molecule in an effector/receptor binding pair; or an antibody or antibody fragment molecule in antigen/antibody or antigen/antibody fragment binding pair, respectively.

"Analyte" or "analyte molecule" refers to a molecule, typically a macromolecule, such as a polynucleotide or polypeptide, whose presence, amount, and/or identity are to be determined. The analyte is one member of a ligand/anti-ligand pair.

"Analyte-specific assay reagent" refers to a molecule effective to bind specifically to an analyte molecule. The reagent is the opposite member of a ligand/anti-ligand binding pair.

An "array of regions on a solid support" is a linear or two-dimensional array of pr ferably discrete

differentiation, or a cell associated with a given pathology or genetic makeup.

II. Method of Microarray Formation

This section describes a method of forming a microarray of analyte-assay regions on a solid support or substrate, where each region in the array has a known amount of a selected, analyte-specific reagent.

Fig. 1 illustrates, in a partially schematic view, a reagent-dispensing device 10 useful in practicing the method. The device generally includes a reagent dispenser 12 having an elongate open capillary channel 14 adapted to hold a quantity of the reagent solution, such as indicated at 16, as will be described below. The capillary channel is formed by a pair of spaced-apart, coextensive, elongate members 12a, 12b which are tapered toward one another and converge at a tip or tip region 18 at the lower end of the channel. More generally, the open channel is formed by at least two elongate, spaced-apart members adapted to hold a quantity of reagent solutions and having a tip region at which aqueous solution in the channel forms a meniscus, such as the concave meniscus illustrated at 20 in Fig. 2A. The advantages of the open channel construction of the dispenser are discussed below.

With continued reference to Fig. 1, the dispenser device also includes structure for moving the dispenser rapidly toward and away from a support surface, for effecting deposition of a known amount of solution in the dispenser on a support, as will be described below with reference to Figs. 2A-2C. In the embodiment shown, this structure includes a solenoid 22 which is activatable to draw a solenoid piston 24 rapidly downwardly, then release th piston, *.g.*, under spring bias, to a normal, raised position, as shown. The

to a selected position with respect to a support surface, placing the dispenser tip directly above the support-surface position at which the reagent is to be deposited. This movement takes place with the dispenser tip in its raised position, as seen in Fig. 2A, where the tip is typically at least several 1-5 mm above the surface of the substrate.

With the dispenser so positioned, solenoid 22 is now activated to cause the dispenser tip to move rapidly toward and away from the substrate surface, making momentary contact with the surface, in effect, tapping the tip of the dispenser against the support surface. The tapping movement of the tip against the surface acts to break the liquid meniscus in the tip channel, bringing the liquid in the tip into contact with the support surface. This, in turn, produces a flowing of the liquid into the capillary space between the tip and the surface, acting to draw liquid out of the dispenser channel, as seen in Fig. 2B.

Fig. 2C shows flow of fluid from the tip onto the support surface, which in this case is a hydrophobic surface. The figure illustrates that liquid continues to flow from the dispenser onto the support surface until it forms a liquid bead 32. At a given bead size, *i.e.*, volume, the tendency of liquid to flow onto the surface will be balanced by the hydrophobic surface interaction of the bead with the support surface, which acts to limit the total bead area on the surface, and by the surface tension of the droplet, which tends toward a given bead curvature. At this point, a given bead volume will have formed, and continued contact of the dispenser tip with the bead, as the dispenser tip is being withdrawn, will have little or no effect on bead volume.

Tabl 1

d	Volume (nl)
20 μm	2×10^{-3}
50 μm	3.1×10^{-2}
100 μm	2.5×10^{-1}
200 μm	2

At a given tip size, bead volume can be reduced in a controlled fashion by increasing surface hydrophobicity, reducing time of contact of the tip with the surface, increasing rate of movement of the tip away from the surface, and/or increasing the viscosity of the medium. Once these parameters are fixed, a selected deposition volume in the desired pl to nl range can be achieved in a repeatable fashion.

After depositing a bead at one selected location on a support, the tip is typically moved to a corresponding position on a second support, a droplet is deposited at that position, and this process is repeated until a liquid droplet of the reagent has been deposited at a selected position on each of a plurality of supports.

The tip is then washed to remove the reagent liquid, filled with another reagent liquid and this reagent is now deposited at each another array position on each of the supports. In one embodiment, the tip is washed and refilled by the steps of (i) dipping the capillary channel of the device in a wash solution, (ii) removing wash solution drawn into the capillary channel, and (iii) dipping the capillary channel into the new reagent solution.

From the foregoing, it will be appreciated that the tweezers-like, open-capillary dispenser tip

III. Automated Apparatus for Forming Arrays

In another aspect, the invention includes an automated apparatus for forming an array of analyte-assay regions on a solid support, where each region in the array has a known amount of a selected, analyte-specific reagent.

The apparatus is shown in planar, and partially schematic view in Fig. 4. A dispenser device 72 in the apparatus has the basic construction described above with respect to Fig. 1, and includes a dispenser 74 having an open-capillary channel terminating at a tip, substantially as shown in Figs. 1 and 2A-2C.

The dispenser is mounted in the device for movement toward and away from a dispensing position at which the tip of the dispenser taps a support surface, to dispense a selected volume of reagent solution, as described above. This movement is effected by a solenoid 76 as described above. Solenoid 76 is under the control of a control unit 77 whose operation will be described below. The solenoid is also referred to herein as dispensing means for moving the device into tapping engagement with a support, when the device is positioned at a defined array position with respect to that support.

The dispenser device is carried on an arm 74 which is threadedly mounted on a worm screw 80 driven (rotated) in a desired direction by a stepper motor 82 also under the control of unit 77. At its left end in the figure screw 80 is carried in a sleeve 84 for rotation about the screw axis. At its other end, the screw is mounted to the drive shaft of the stepper motor, which in turn is carried on a sleeve 86. The dispenser device, worm screw, the two sleeves mounting the w rm screw, and the stepper motor used in moving th device in the "x" (horizontal) directi n in the

dispensing device at a selected array position with respect to a support.

A holder 102 in the apparatus functions to hold a plurality of supports, such as supports 104 on which the microarrays of regent regions are to be formed by the apparatus. The holder provides a number of recessed slots, such as slot 106, which receive the supports, and position them at precise selected positions with respect to the frame bars on which the dispenser moving means is mounted.

As noted above, the control unit in the device functions to actuate the two stepper motors and dispenser solenoid in a sequence designed for automated operation of the apparatus in forming a selected microarray of reagent regions on each of a plurality of supports.

The control unit is constructed, according to conventional microprocessor control principles, to provide appropriate signals to each of the solenoid and each of the stepper motors, in a given timed sequence and for appropriate signalling time. The construction of the unit, and the settings that are selected by the user to achieve a desired array pattern, will be understood from the following description of a typical apparatus operation.

Initially, one or more supports are placed in one or more slots in the holder. The dispenser is then moved to a position directly above a well (not shown) containing a solution of the first reagent to be dispensed on the support(s). The dispenser solenoid is actuated now to lower the dispenser tip into this well, causing the capillary channel in the dispenser to fill. Motors 82, 100 are now actuated to position the dispenser at a selected array position at the first of the supports. Solenoid actuation of the dispenser is

This section describes embodiments of a substrate having a microarray of biological polymers carried on the substrate surface. Subsection A describes a multi-cell substrate, each cell of which contains a microarray, and preferably an identical microarray, of distinct biopolymers, such as distinct polynucleotides, formed on a porous surface. Subsection B describes a microarray of distinct polynucleotides bound on a glass slide coated with a polycationic polymer.

A. Multi-Cell Substrate

Fig. 9 illustrates, in plan view, a substrate 110 constructed according to the invention. The substrate has an 8 x 12 rectangular array 112 of cells, such as cells 114, 116, formed on the substrate surface. With reference to Fig. 10, each cell, such as cell 114, in turn supports a microarray 118 of distinct biopolymers, such as polypeptides or polynucleotides at known, addressable regions of the microarray. Two such regions forming the microarray are indicated at 120, and correspond to regions, such as regions 42, forming the microarray of distinct biopolymers shown in Fig. 3.

The 96-cell array shown in Fig. 9 has typically array dimensions between about 12 and 244 mm in width and 8 and 400 mm in length, with the cells in the array having width and length dimension of 1/12 and 1/8 the array width and length dimensions, respectively, *i.e.*, between about 1 and 20 in width and 1 and 50 mm in length.

The construction of substrate is shown cross-sectionally in Fig. 11, which is an enlarged sectional view taken along view line 124 in Fig. 9. The substrate includes a water-impermeable backing 126, such as a glass slide or rigid polymer sheet. Formed on the surface of the backing is a water-permeable film

In alternative embodiments, the barrier element can be a wax-based material or a thermoset material such as epoxy. The barrier material can also be a UV-curing polymer which is exposed to UV light after being printed onto the solid support. The barrier material may also be applied to the solid support using printing techniques such as silk-screen printing. The barrier material may also be a heat-seal stamping of the porous solid support which seals its pores and forms a water-impervious barrier element. The barrier material may also be a shallow grid which is laminated or otherwise adhered to the solid support.

In addition to plastic-backed nitrocellulose, the solid support can be virtually any porous membrane with or without a non-porous backing. Such membranes are readily available from numerous vendors and are made from nylon, PVDF, polysulfone and the like. In an alternative embodiment, the barrier element may also be used to adhere the porous membrane to a non-porous backing in addition to functioning as a barrier to prevent cross contamination of the assay reagents.

In an alternative embodiment, the solid support can be of a non-porous material. The barrier can be printed either before or after the microarray of biomolecules is printed on the solid support.

As can be appreciated, the cells formed by the grid lines and the underlying backing are water-impermeable, having side barriers projecting above the porous film in the cells. Thus, defined-volume samples can be placed in each well without risk of cross-contamination with sample material in adjacent cells. In Fig. 11, defined volumes samples, such as sample 134, are shown in the cells.

As noted above, each well contains a microarray of distinct biopolymers. In one general embodiment, th

Each assay may be conducted in an "open-face" format where no further sealing step is required, since the hybridization solution will be kept properly hydrated by the water vapor in the humid chamber. At the conclusion of the incubation step, the entire solid support containing the numerous microarrays is rinsed quickly enough to dilute the assay reagents so that no significant cross contamination occurs. The entire solid support is then reacted with detection reagents if needed and analyzed using standard colorimetric, radioactive or fluorescent detection means. All processing and detection steps are performed simultaneously to all of the microarrays on the solid support ensuring uniform assay conditions for all of the microarrays on the solid support.

B. Glass-Slide Polynucleotide Array

Fig. 5 shows a substrate 136 formed according to another aspect of the invention, and intended for use in detecting binding of labeled polynucleotides to one or more of a plurality distinct polynucleotides. The substrate includes a glass substrate 138 having formed on its surface, a coating of a polycationic polymer, preferably a cationic polypeptide, such as polylysine or polyarginine. Formed on the polycationic coating is a microarray 140 of distinct polynucleotides, each localized at known selected array regions, such as regions 142.

The slide is coated by placing a uniform-thickness film of a polycationic polymer, e.g., poly-l-lysine, on the surface of a slide and drying the film to form a dried coating. The amount of polycationic polymer added is sufficient to form at least a monolayer of polymers on the glass surface. The polymer film is bound to surfac via electrostatic binding between

density arrays of this type, where each region is formed of a well-defined amount of deposited material, can be achieved in accordance with the microarray-forming method described in Section II.

Also in a preferred embodiments, the polynucleotides have lengths of at least about 50 bp, *i.e.*, substantially longer than oligonucleotides which can be formed in high-density arrays by various in situ synthesis schemes.

V. Utility

Microarrays of immobilized nucleic acid sequences prepared in accordance with the invention can be used for large scale hybridization assays in numerous genetic applications, including genetic and physical mapping of genomes, monitoring of gene expression, DNA sequencing, genetic diagnosis, genotyping of organisms, and distribution of DNA reagents to researchers.

For gene mapping, a gene or a cloned DNA fragment is hybridized to an ordered array of DNA fragments, and the identity of the DNA elements applied to the array is unambiguously established by the pixel or pattern of pixels of the array that are detected. One application of such arrays for creating a genetic map is described by Nelson, *et al.* (1993). In constructing physical maps of the genome, arrays of immobilized cloned DNA fragments are hybridized with other cloned DNA fragments to establish whether the cloned fragments in the probe mixture overlap and are therefore contiguous to the immobilized clones on the array. For example, Lehrach, *et al.*, describe such a process.

The arrays of immobilized DNA fragments may also be used for genetic diagnostics. To illustrate, an array containing multiple forms f a mutated gene or g nes can be probed with a labeled mixture f a

sheet of plastic-backed nitrocellulose where each microarray could contain, for example, 100 DNA fragments representing all known mutations of a given gene. The region of interest from each of the DNA samples from 96 patients could be amplified, labeled, and hybridized to the 96 individual arrays with each assay performed in 100 microliters of hybridization solution. The approximately 1 thick silicone rubber barrier elements between individual arrays prevent cross contamination of the patient samples by sealing the pores of the nitrocellulose and by acting as a physical barrier between each microarray. The solid support containing all 96 microarrays assayed with the 96 patient samples is incubated, rinsed, detected and analyzed as a single sheet of material using standard radioactive, fluorescent, or colorimetric detection means (Maniatas, *et al.*, 1989). Previously, such a procedure would involve the handling, processing and tracking of 96 separate membranes in 96 separate sealed chambers. By processing all 96 arrays as a single sheet of material, significant time and cost savings are possible.

The assay format can be reversed where the patient or organism's DNA is immobilized as the array elements and each array is hybridized with a different mutated allele or genetic marker. The gridded solid support can also be used for parallel non-DNA ELISA assays. Furthermore, the invention allows for the use of all standard detection methods without the need to remove the shallow barrier elements to carry out the detection step.

In addition to the genetic applications listed above, arrays of whole cells, peptides, enzymes, antibodies, antigens, receptors, ligands, phospholipids, polymers, drug cogener preparations or

lambda clones was rehydrated in 15 μl of 3 × SSC in preparation for spotting onto the glass.

The micro arrays were fabricated on microscope slides which were coated with a layer of poly-l-lysine (Sigma). The automated apparatus described in Section IV loaded 1 μl of the concentrated lambda clone PCR product in 3 × SSC directly from 96 well storage plates into the open capillary printing element and deposited ~5 nl of sample per slide at 380 micron spacing between spots, on each of 40 slides. The process was repeated for all 864 samples and 8 control spots. After the spotting operation was complete, the slides were rehydrated in a humid chamber for 2 hours, baked in a dry 80° vacuum oven for 2 hours, rinsed to remove un-absorbed DNA and then treated with succinic anhydride to reduce non-specific adsorption of the labeled hybridization probe to the poly-l-lysine coated glass surface. Immediately prior to use, the immobilized DNA on the array was denatured in distilled water at 90° for 2 minutes.

For the pooled chromosome experiment, the 16 chromosomes of *Saccharomyces cerevisiae* were separated in a CHEF agarose gel apparatus (Biorad, Richmond, CA). The six largest chromosomes were isolated in one gel slice and the smallest 10 chromosomes in a second gel slice. The DNA was recovered using a gel extraction kit (Qiagen, Chatsworth, CA). The two chromosome pools were randomly amplified in a manner similar to that used for the target lambda clones. Following amplification, 5 micrograms of each of the amplified chromosome pools were separately random-primer labeled using Klenow polymerase (Amersham, Arlington Heights, IL) with a lissamine conjugated nucleotide analog (Dupont NEN, Bost n, MA) for the pool containing the six largest chromosomes, and with a fluorescein

The physical map locations of the genomic DNA fragments contained in each of the clones used as array elements have been previously determined by Olson and co-workers (Riles, *et al.*) allowing for the automatic generation of the color karyotype shown in Figure 7. The color of a chromosomal section on the karyotype corresponds to the color of the array element containing the clone from that section. The black regions of the karyotype represent false negative dark spots on the array (10%) or regions of the genome not covered by the Olson clone library (90%). Note that the largest six chromosomes are mainly red while the smallest ten chromosomes are mainly green matching the original CHEF gel isolation of the hybridization probe. Areas of the red chromosomes containing green spots and vice-versa are probably due to spurious sample tracking errors in the formation of the original library and in the amplification and spotting procedures.

The yeast genome arrays have also been probed with individual clones or pools of clones that are fluorescently labeled for physical mapping purposes. The hybridization signals of these clones to the array were translated into a position on the physical map of yeast.

Example 2

Total cDNA Hybridized to Micro Arrays of cDNA Clones with Two-Color Fluorescent Detection

24 clones containing cDNA inserts from the plant *Arabidopsis* were amplified using PCR. Salt was added to the purified PCR products to a final concentration of 3 × SSC. The cDNA clones were spotted on poly-l-lysine coated microscope slides in a manner similar to Example 1. Among the cDNA clones was a clone

transcription factor in th green-labeled wild type *Arabidopsis*.

An advantage of the microarray hybridization format for gene expression studies is the high partial concentration of each cDNA species achievable in the 10 microliter hybridization reaction. This high partial concentration allows for detection of rare transcripts without the need for PCR amplification of the hybridization probe which may bias the true genetic representation of each discrete cDNA species.

Gene expression studies such as these can be used for genomics research to discover which genes are expressed in which cell types, disease states, development states or environmental conditions. Gene expression studies can also be used for diagnosis of disease by empirically correlating gene expression patterns to disease states.

Example 3

Multiplexed Colorimetric Hybridization on a Gridded Solid Support

A sheet of plastic-backed nitrocellulose was gridded with barrier elements made from silicone rubber according to the description in Section IV-A. The sheet was soaked in 10 × SSC and allowed to dry. As shown in Fig. 12, 192 M13 clones each with a different yeast inserts were arrayed 400 microns apart in four quadrants of the solid support using the automated device described in Section III. The bottom left quadrant served as a negative control for hybridization while each of the other three quadrants was hybridized simultaneously with a different oligonucleotide using the open-face hybridization technology described in Section IV-A. The first two and last four elements of

IT IS CLAIMED:

1. A method of forming a microarray of analyte-assay regions on a solid support, where each region in the array has a known amount of a selected, analyte-specific reagent, said method comprising,

(a) loading a solution of a selected analyte-specific reagent in a reagent-dispensing device having an elongate capillary channel (i) formed by spaced-apart, coextensive elongate members, (ii) adapted to hold a quantity of the reagent solution and (iii) having a tip region at which aqueous solution in the channel forms a meniscus,

(b) tapping the tip of the dispensing device against a solid support at a defined position on the surface, with an impulse effective to break the meniscus in the capillary channel and deposit a selected volume of solution on the surface, and

(c) repeating steps (a) and (b) until said array is formed.

2. The method of claim 1, wherein said tapping is carried out with an impulse effective to deposit a selected volume in the volume range between 0.01 to 100 nl.

3. The method of claim 1, wherein said channel is formed by a pair of spaced-apart tapered elements.

4. The method of claim 1, for forming a plurality of such arrays, wherein step (b) is applied to a selected position on each of a plurality of solid supports at each repeat cycle proceeding step (c).

7. The apparatus of claim 6, wherein said dispensing means is effective to move said dispensing device against a support with an impulse effective to deposit a selected volume in the volume range between 0.01 to 100 nl.

8. The apparatus of claim 6, wherein said channel is formed by a pair of spaced-apart tapered elements.

9. The apparatus of claim 6, wherein the control means operates to (i) place the dispensing device at a loading station, (ii) move the capillary channel in the device into a selected reagent at the loading station, to load the dispensing device with the reagent, and (iii) dispense the reagent at a defined array position on each of the supports on said holder.

10. The apparatus of claim 6, wherein the control device further operates, at the end of a dispensing cycle, to wash the dispensing device by (i) placing the dispensing device at a washing station, (ii) moving the capillary channel in the device into a wash fluid, to load the dispensing device with the fluid, and (iii) remove the wash fluid prior to loading the dispensing device with a fresh selected reagent.

11. The apparatus of claim 6, wherein said device is one of a plurality of such devices which are carried on the arm for dispensing different analyte assay reagents at selected spaced array positions.

12. A substrate with a surface having a microarray of at least 10^3 distinct polynucleotide or polypeptide biopolymers per 1 cm^2 surface area, each

a non-porous, glass substrate,

a coating of a cationic polymer on said substrate, and

an array of distinct polynucleotides to said coating, where each biopolymer is disposed at a separate, defined position in a surface array of biopolymers.

18. A method of detecting differential expression of each of a plurality of genes in a first cell type with respect to expression of the same genes in a second cell types, said method comprising

producing fluorescence-labeled cDNA's from mRNA's isolated from the two cells types, where the cDNA's from the first and second cells are labeled with first and second different fluorescent reporters,

adding a mixture of the labeled cDNA's from the two cell types to an array of polynucleotides representing a plurality of known genes derived from the two cell types, under conditions that result in hybridization of the cDNA's to complementary-sequence polynucleotides in the array; and

examining the array by fluorescence under fluorescence excitation conditions in which (i) polynucleotides in the array that are hybridized predominantly to cDNA's derived from one of the first and second cell types give a distinct first or second fluorescence emission color, respectively, and (ii) polynucleotides in the array that are hybridized to substantially equal numbers of cDNA's derived from the first and second cell types give a distinct combined fluorescence emission color, respectively,

wherein the relative expression of known genes in the two cell types can be determined by the observed fluorescence emission color of each spot.

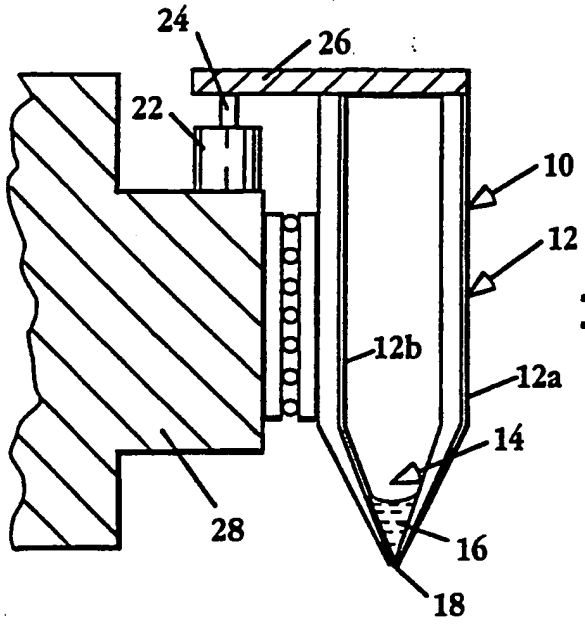

Fig. 1

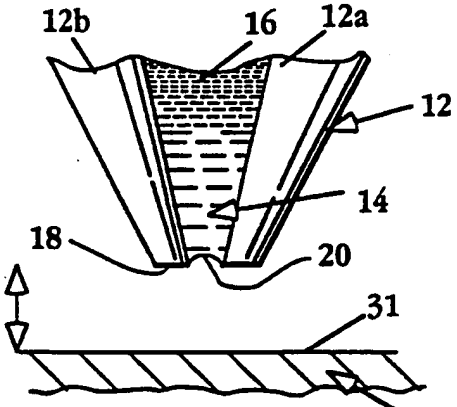

Fig. 2A

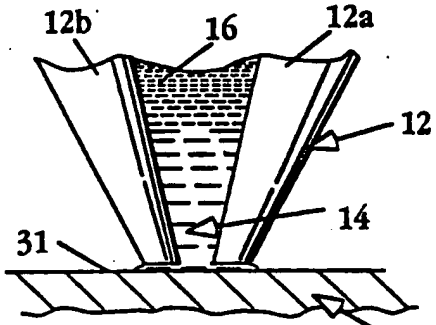

Fig. 2B

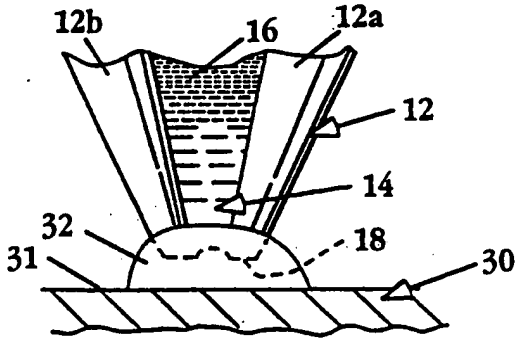

Fig. 2C

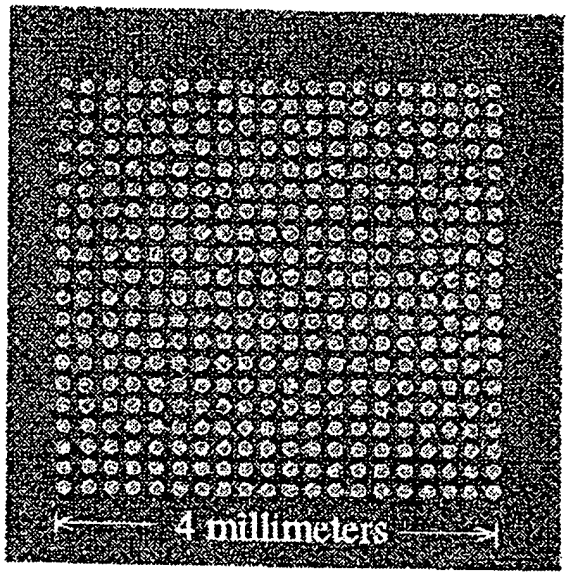

Fig. 5

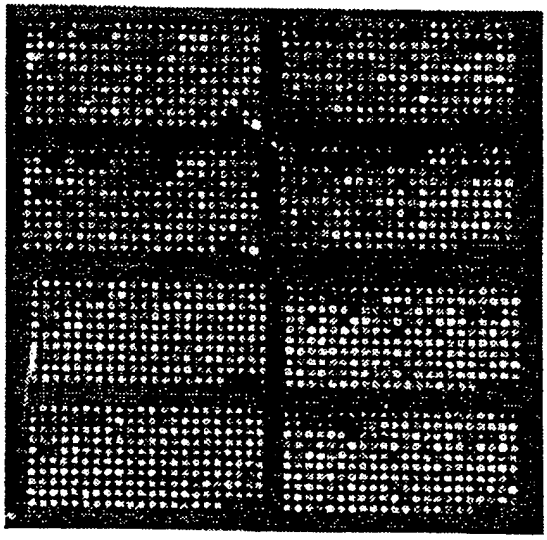

Fig. 6

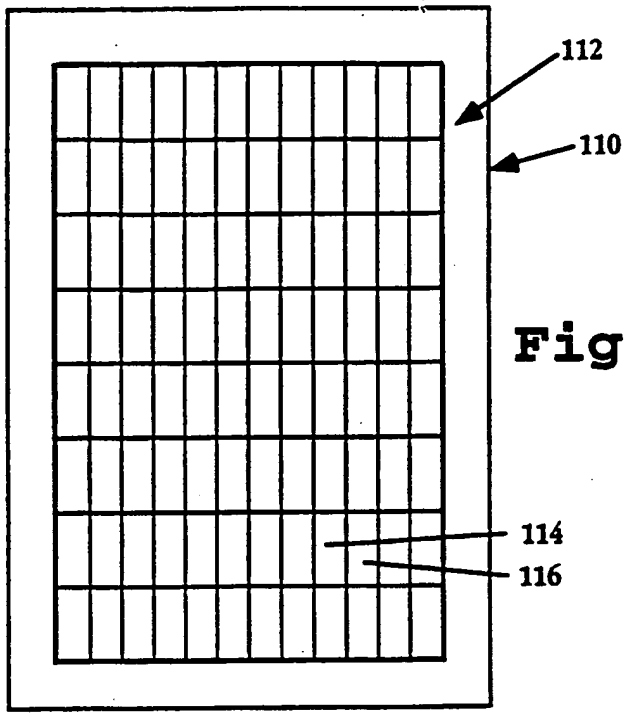

Fig. 9

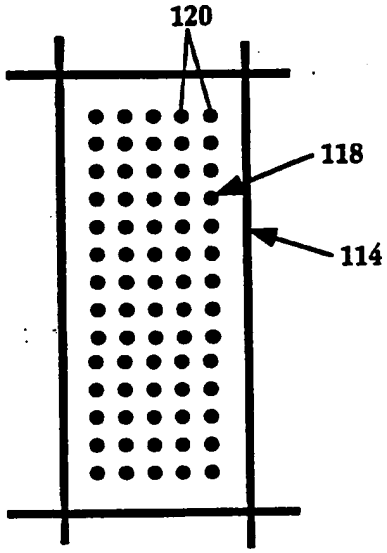

Fig. 10

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/07659

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :G01N 33/543, 33/68

US CL :435/6; 436/518

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 422/57; 435/4.6,973; 436/518,524,527,531,805,809

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A,P	US, A, 5,338,688 (DEEG ET AL) 16 August 1994, see entire document	1-17
A	US, A, 5,204,268 (MATSUMOTO) 20 April 1993, see entire document.	6-11
A	US, A, 4,071,315 (CHATEAU) 31 January 1978, see entire document.	12-17
A	US, A, 5,100,777 (CHANG) 31 March 1992, see entire document.	12-17
A	US, A, 5,200,312 (OPRANDY) 06 April 1993, see entire document.	12-17

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 15 SEPTEMBER 1995	Date of mailing of the international search report 06 OCT 1995
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer CHRISTOPHER CHIN Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*